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## Transcriptomic analyses of Onecut1 and Onecut2 deficient retinas

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## Data in Brief

Transcriptomic analyses of *Onecut1* and *Onecut2* deficient retinas

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## ABSTRACT

In this article, we further explore the data generated for the research article “*Onecut1* and *Onecut2* play critical roles in the development of the mouse retina”. To better understand the functionality of the *Onecut* family of transcription factors in retinogenesis, we investigated the retinal transcriptomes of developing and mature mice to identify genes with differential expression. This data article reports the full transcriptomes resulting from these experiments and provides tables detailing the differentially expressed genes between wildtype and *Onecut1* or *2* deficient retinas. The raw array data of our transcriptomes as generated using Affymetrix microarrays are available on the NCBI Gene Expression Omnibus (GEO) browser (Reference number GSE57917 and GSE57918).

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Specifications	
Organism/cell line/tissue	Mouse
Sex	N/A
Sequencer or array type	Affymetrix GeneChip Mouse Genome 430 2.0
Data format	Raw and analyzed
Experimental factors	<i>Onecut1</i> WT and KO, <i>Onecut2</i> WT and KO
Experimental features	Transcriptomes from isolated retinas of <i>Onecut1</i> or <i>Onecut2</i> KO mice and age-matched littermates were compared to determine the effects of <i>Onecut</i> transcription factor deficiency during retinal development.
Consent	Level of consent allowed for reuse if applicable
Sample source location	Ames, Iowa, USA

## Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57917>.  
<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57918>.

## Experimental design, materials and methods, data

## RNA isolation

To determine the retinal transcriptomic changes that result from *Onecut1* and *Onecut2* deficiencies, we isolated retinas from embryonic day (E)14.5 *Onecut1*-KO retinas and their WT littermates or adult

*Onecut2*-KO and WT littermates [1]. TRI-reagent (Sigma) was employed to isolate RNA according to manufacturer's instructions. Briefly, the isolated retinas were deposited into 1 ml of TRI-reagent. At this point, we routinely freeze our samples in TRI-reagent at  $-80^{\circ}\text{C}$  and find that better RNA yields are achieved if you do freeze the samples. The samples are then thawed and pipetted repeatedly to homogenize. After letting the samples incubate at room temperature for 5 min, 100  $\mu\text{l}$  of 1-bromo-3-chloropropane was added and the sample was vigorously shaken for 15 min. on a vortex. After standing for 10 min at room temperature, the samples were centrifuged for 15 min at  $12,000 \times g$  at  $4^{\circ}\text{C}$ . The aqueous phase was isolated and 500  $\mu\text{l}$  of isopropanol was added and mixed by inversion (4–6 times). The samples were allowed to stand at room temperature for 10 min and then were centrifuged again for 15 min at  $12,000 \times g$  at  $4^{\circ}\text{C}$ . The samples were washed with 500  $\mu\text{l}$  75% ethanol, centrifuged further for 5 min at  $12,000 \times g$  at  $4^{\circ}\text{C}$ , and air-dried for 10 min. The RNA samples were then resuspended in 25  $\mu\text{l}$  of nuclease-free water and stored at  $-80^{\circ}\text{C}$ . Concentrations were determined using a Nanodrop.

## Microarray preparation

For reverse transcription, 400 ng of total RNA was added to the First Strand Enzyme Master Mix in the MessageAmp III RNA Amplification Kit (Ambion). This 10  $\mu\text{l}$  reaction was mixed and then incubated at  $42^{\circ}\text{C}$  for 2 h in a thermocycler. After incubation, 20  $\mu\text{l}$  of Second Strand Master Mix was added to each sample and the samples were mixed. Second strand synthesis was performed for 1 h at  $16^{\circ}\text{C}$  in a thermocycler. The samples were then heated to  $65^{\circ}\text{C}$ . At this point,

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the samples could be stored at  $-20^{\circ}\text{C}$  before the *in vitro* transcription reaction. A T7-based *in vitro* transcription reaction was performed by adding 30  $\mu\text{l}$  of the T7 IVT Master Mix to the second strand synthesis samples and incubating the tubes in a thermocycler at  $40^{\circ}\text{C}$  for 8 h. The samples were then stored at  $-20^{\circ}\text{C}$  overnight. Amplified RNA (aRNA) purification was then performed by first adding 40  $\mu\text{l}$  of nuclease-free water to bring each sample to a total volume of 100  $\mu\text{l}$  before adding 350  $\mu\text{l}$  of aRNA binding buffer. This was immediately followed by the addition of 250  $\mu\text{l}$  of ACS grade 100% ethanol to each sample. Samples were gently triturated three times to mix thoroughly before being applied to the center of the filter of an aRNA filter cartridge placed in an aRNA collection tube. After the flow-through was discarded, the aRNA filter cartridge was washed with 650  $\mu\text{l}$  of wash buffer and spun at  $10,000 \times g$ . The aRNA was then eluted into a fresh microcentrifuge tube using 100  $\mu\text{l}$  of nuclease-free  $\text{dH}_2\text{O}$  that was preheated to  $60^{\circ}\text{C}$  for 15 min. 10  $\mu\text{g}$  of this aRNA sample was fragmented in a 40  $\mu\text{l}$  reaction by heating to  $94^{\circ}\text{C}$  for 35 min and then hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 arrays at Iowa State University's GeneChip Facility. Standard Affymetrix hybridization protocols were utilized.

#### *R workflow for microarray normalization*

Analysis of microarray data was performed using the Bioconductor Affy package for R [2]. Data was background adjusted and normalized using Mas5 and  $\log(2)$  transformed.

```
> library("affy")
> raw<-ReadAffy()
> eset.mas5<-mas5(raw)
> exprSet.nologs<-exprs(eset.mas5)
> exprSet<-log(exprSet.nologs,2)
> write.table(exprSet, file="output.txt", quote=F, sep="\t")
```

Analyses of differential expression were limited to genes whose mean expression level among either  $n = 3$  WT or KO retinas exceeded a log-transformed value of 7. Two-tailed t-tests resulting in p-values of less than 0.05 indicated significant differential expression.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gdata.2015.03.010>.

#### References

- [1] J.J. Goetz, et al., *Onecut1 and Onecut2 play critical roles in the development of the mouse retina*. PLoS ONE 9 (10) (2014) e110194.
- [2] L. Gautier, et al., *affy-analysis of Affymetrix GeneChip data at the probe level*. Bioinformatics 20 (3) (2004) 307–315.